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Redescription of *Lutzomyia* (*Lutzomyia*) *renei* Martins, Falcão & Silva, 1957 (Diptera: Psychodidae: Phlebotominae)

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Abstract

The male genitalia of *Lutzomyia* (*Lutzomyia*) *renei* (Martins, Falcão & Silva, 1957) have four bristles, three fine and one semi-foliaceous, inserted basomesally on the gonocoxite. Nonetheless, in the original description and in other taxonomic studies, these bristles have been illustrated and described in varying formats. In order to clarify the morphology of this species, both sexes are here redescribed based on three males and one female from the type series. A lectotype and two paralectotypes are here designated.

Key words: American sand flies, Brazil, morphology, taxonomy

Introduction

Lutzomyia (*Lutzomyia*) *renei* (Martins, Falcão & Silva, 1957) (Psychodidae, Phlebotominae, Phlebotomini, Lutzomyiina) is one of 21 species in the subgenus (Galati 2015). Martins *et al*. (1957) described the male of this species based on six specimens and designated them as "cotypes". Lapinha Cave, Lagoa Santa municipality, Minas Gerais state, Brazil was listed as the type locality. In the same year, Sherlock (1957) obtained males of *Lu. renei* reared in the laboratory from eggs laid by females collected in the type locality. He described the female based on one specimen. In addition, the immature stages were described and details of the life cycle, phenology, anthropophilic behavior and hourly activity, as observed under experimental and natural conditions, were provided (Sherlock 1957).

Males of species included in subgenus *Lutzomyia* sensu stricto (Galati 2003, 2015) have a cluster of two to five bristles on the mesal surface of each gonocoxite near its base. These bristles may be fine (as wide as the genital filaments), semi-foliaceous (twice as wide as the fine bristles) or foliaceous (three or more times as wide as the fine bristles). *Lutzomyia renei*, as well as eleven other species: *Lu. alencari*, *Lu. battistinii, Lu. bicornuta*, *Lu. cavernicola*, *Lu. cruzi*, *Lu. dispar*, *Lu. gaminarai*, *Lu. ischnacantha*, *Lu. ischyracantha*, *Lu. longipalpis*, *Lu. pseudolongipalpis* and *Lu. souzalopesi* have four bristles in this cluster. Martins *et al*. (1957) illustrated the four bristles of *Lu. renei* as one fine one and three semi-foliaceous. Forattini (1973) maintained the same arrangement. Theodor (1965), in his proposal for classification of the American Phlebotominae, illustrated only the gonostylus and Young & Duncan (1994) drew all bristles as semi-foliaceous. Despite various descriptions of this species, the exact morphology of the gonocoxal bristles in *Lu. renei* has not yet been described and illustrated. For the female of this species, some structures of the cibarium have not been accurately reproduced.

Inadequate descriptions and illustrations may lead to the misidentification of species; thus, the present study aims to present a detailed morphological and morphometric description of both sexes of *Lu. renei* in order to provide a greater number of characteristics for its differentiation from other closely-related species.

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Material and methods

As noted above, Martins *et al.* (1957) described *Lu. renei* from six males. Collection dates of these specimens are as follows:10-III-1955 (one specimen), 01-X-1955 (one specimen), 22-X-1955 (two specimens) and 24-XI-1955 (two specimens), all are labeled as "cotypes". According to the International Code of Zoological Nomenclature (ICZN 1999): "The valid designation of a lectotype permanently deprives all other specimens that were formerly syntypes of that nominal taxon of the status of syntype [Art. 73.2.2]; those specimens then become paralectotypes" [Art. 74]. Syntypes have the same taxonomic value as "cotypes" [Art. 72.4.6]. Here we provide a designation of one lectotype and two paralectotypes of *Lu. renei*, excluding the female.

In addition, we examined 2 ♂ from the type locality (E-3220 and E-3221) and 2 ♂ (E-2424 and E-3314) from Pirapora municipality, Minas Gerais state, deposited in the "Coleção de Referência da Faculdade de Saúde Pública" (FSP-USP). The original male description (Martins *et al.* 1957) and female description (Sherlock 1957) as well as other literature were consulted for the present redescription and to determine current geographical distribution.

For the majority of the morphological characters, the terminology adopted follows that of Galati (2003). However, for some characters of the male terminalia and the palpus we are adopting Cumming & Wood (2009) but, in this case, the terminology of Galati (2003) is given between parentheses. Abbreviations of generic names follow Marcondes (2007).

Drawings were made with the aid of an Olympus camera lucida. Measurements were taken with a Zeiss ocular micrometer calibrated using a standard Zeiss scale. Conversion of the micrometer readings was made using objective lens (5X) = 196 µm, (10X) = 100 µm and (40X) = 26 µm. All measurements are given in micrometers (µm). In the redescription, measurements given outside the parentheses correspond to the male "cotype" collected on 10-III-1955, while those inside the parentheses are for the other two male "cotypes" collected on 24-XI-1955 (Martins et al. 1957); the female measured is that described by Sherlock (1957). The interocular distance and the width of the head and the eye of all specimens were not measured because they are mounted on lateral position (drawings not presented here).

Analyses were based on the following characters: length and width of the head and eyes; interocular distance; length of clypeus; labrum-epipharynx; flagellomeres (F) I, II, III, FXIII and FXIV, and palp segments (P) I, II, III, IV and V; length and width of wing and length of some alar veins (*alpha*, *beta*, *gamma*, *delta*, *pi* and R_5). Regarding male terminalia: length and width of gonocoxite and epandrium (lateral lobes), length of gonostylus, dorsal and ventral margin of paramere, sperm pump (ejaculatory pump) and aedeagus (genital filaments). For the female genitalia, length and width of the spermathecae as well as individual and common spermathecal ducts were measured.

Besides morphological characters of the males and females, the distribution of the thoracic pigmentation of the taxa was compared and classified as: intense (brown), low intensity (straw), or absent (off-white) (Caillard *et al*. 1986, Andersen 2010).

Results

Lutzomyia (*Lutzomyia*) *renei* (Martins, Falcão & Silva)

(Figs 1–4)

Phlebotomus renei Martins, Falcão & Silva, 1957: 321. Type series: six males "cotypes": Lapinha Cave, Lagoa Santa municipality, Minas Gerais state, Brazil, July/September1957, F. R. Bastos coll. (Revista Brasileira de Malariologia e Doenças Tropicais); Sherlock 1957: 547 (description of female and immature forms); Coelho 1962: 102 (exp. *Leishmania* infection); Coelho & Falcão 1962: 220 (exp. *Leishmania braziliensis* infection); Sherlock & Pessôa 1964: 332 (sample methods).

Lutzomyia renei Barretto1962: 92 (cat., comb.); Theodor 1965: 181 (cat., male fig.); Coelho *et al.* 1967a–d (exp. *Leishmania* infection); Forattini 1971: 100; 1973: 266 (cat., figs, tax.); Christensen *et al.* 1972: 55 (exp. *Leishmania* infection); Lewis *et al*. 1977: 325 (cat.); Martins *et al*. 1978: 25 (cat., distribution); Galati *et al*. 1985: 266 (tax. male); Killick-Kendrick 1986: 135 (cat., *Leishmania* infection); Gontijo *et al*. 1987: 445 (exp. *Leishmania* infection); Artemiev 1991: 73 (cat.); Young & Duncan 1994: 53 (cat., figs, tax., distribution); Galati & Nunes 1999; 280 (tax.); Alves *et al*. 2003: 121 (biological cycle); Galati 2003: 14 (cat., tax., distr.).

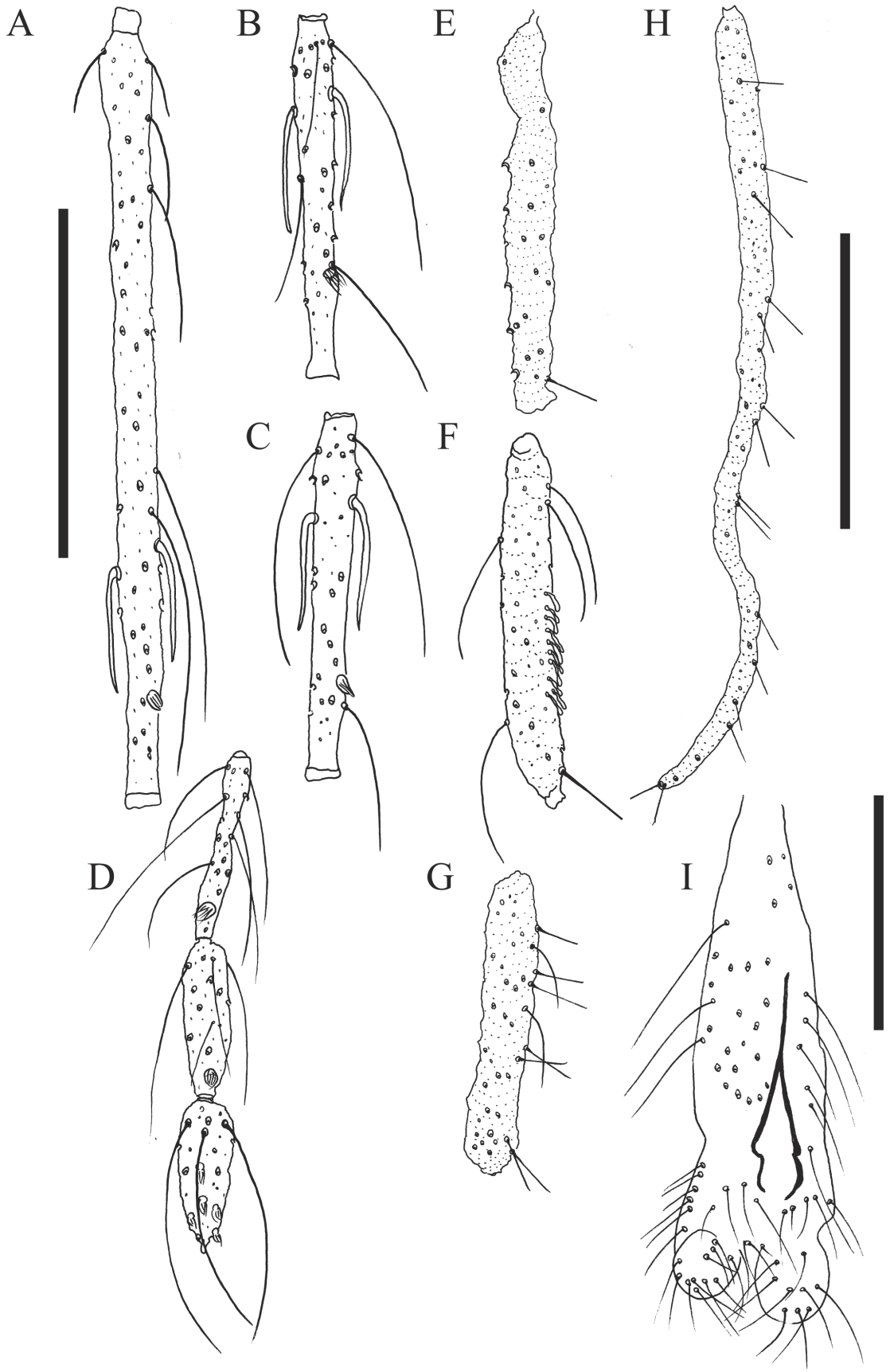

FIGURE 1. Male of *Lutzomyia* (*Lu.*) *renei* (Martins, Falcão & Silva, 1957). **A**. Flagellomere I. **B**. Flagellomere II. **C**. Flagellomere III. **D**. Flagellomeres XII, XIII and XIV. **E**. Palpus I and II. **F**. Palpus III. **G**. Palpus IV. **H**. Palpus V. **I**. Labial fork (Bar: 100µm).

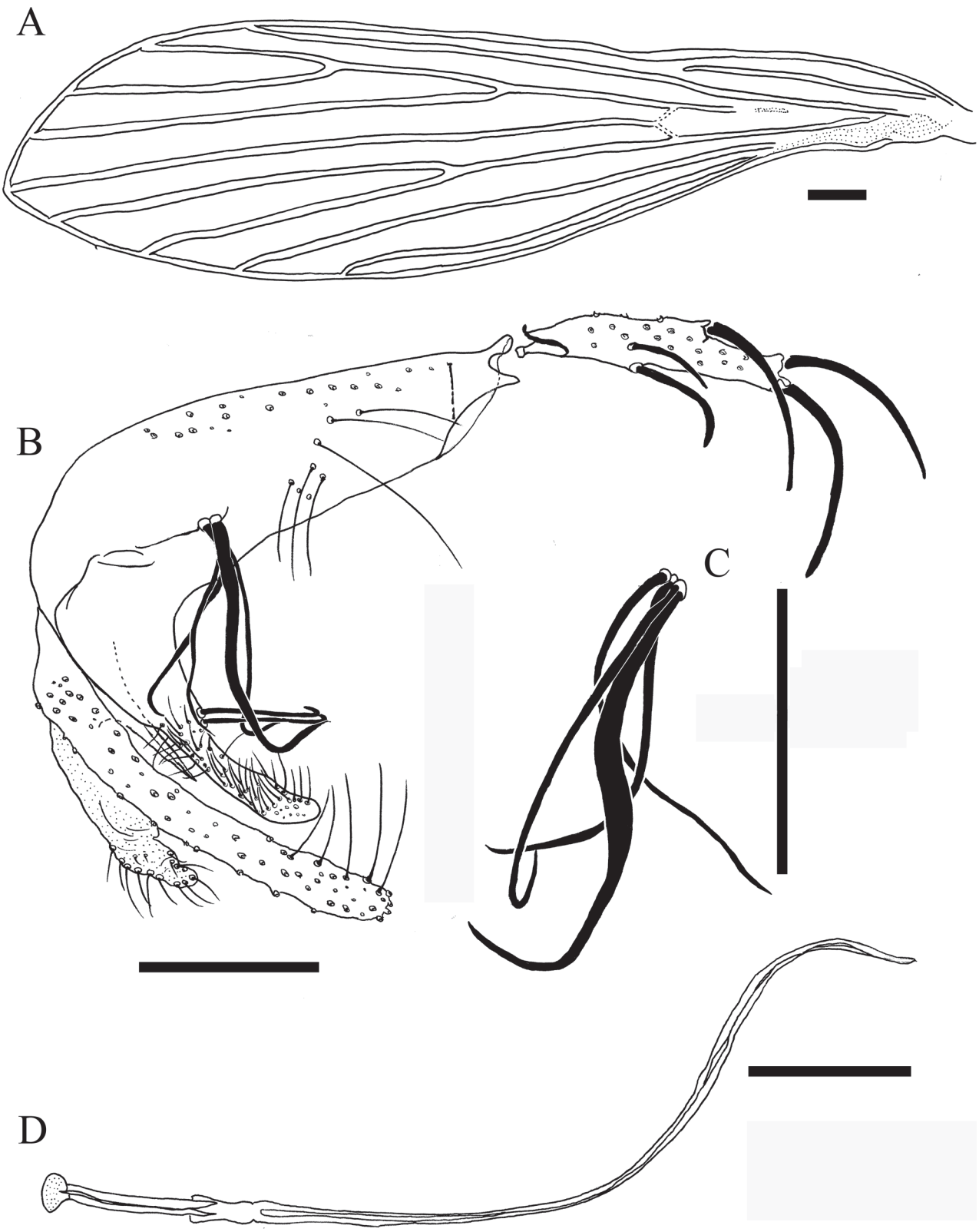

FIGURE 2. Male *Lutzomyia* (*Lu.*) *renei* (Martins, Falcão & Silva, 1957). **A**. Wing (Bar: 200µm). **B**. Terminalia (Bar: 100µm). **C**. Cluster of three fine bristles and one semi-foliaceous bristle implanted on surface of gonocoxite. **D**. Apodeme, sac and genital filaments (Bar: 100µm).

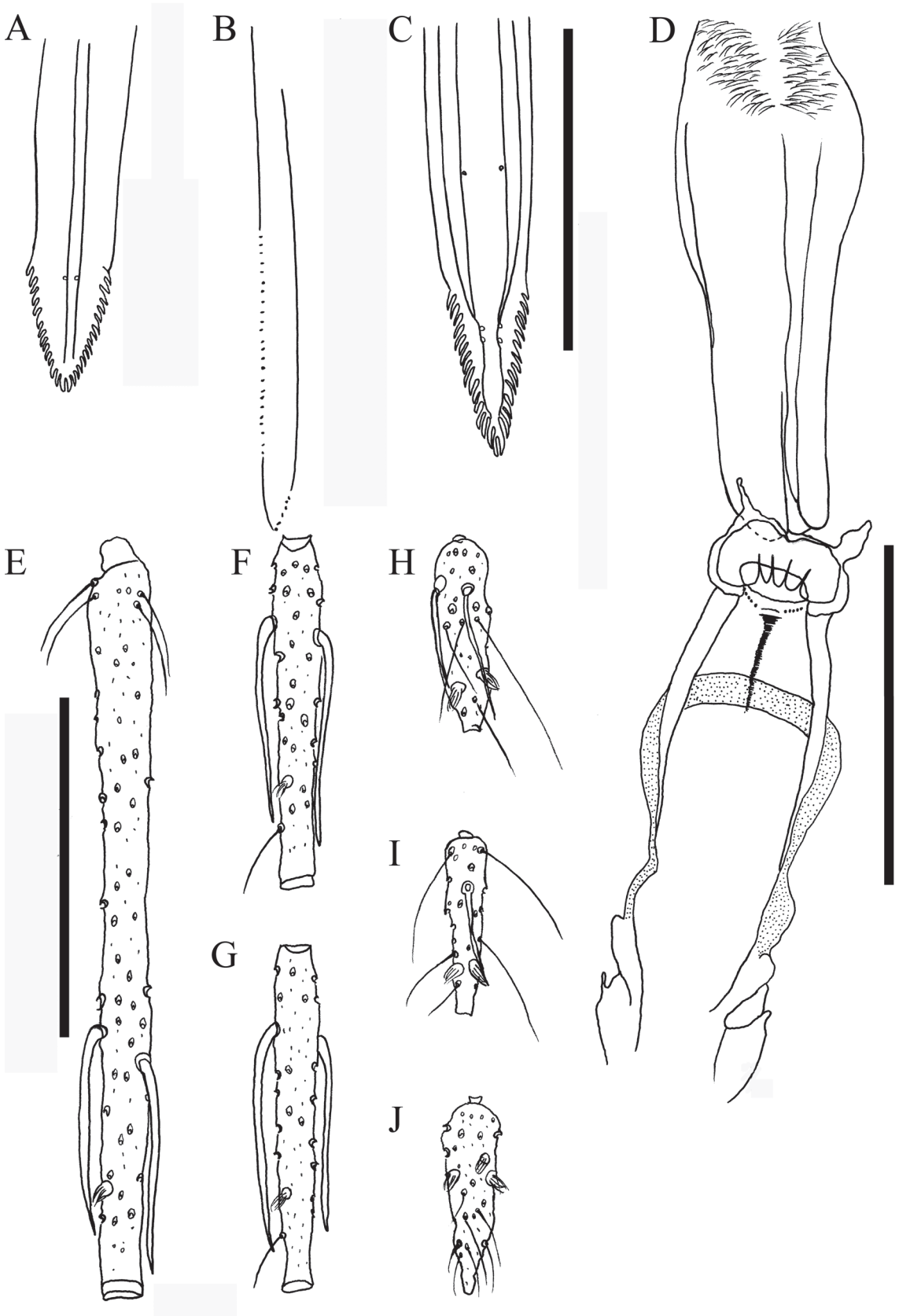

FIGURE 3. Female of *Lutzomyia* (*Lu.*) *renei* (Martins, Falcão & Silva, 1957). **A**. Apical region of hypopharynx. **B**. Apical region of lacinia of the maxilla. **C**. Apical region of labrum-epipharynx. **D**. Cibarium. **E**) Flagellomere I. **F**. Flagellomere II. **G**. Flagellomere III. **H**. Flagellomere XII. **I**. Flagellomere XIII. **J**. Flagellomere XIV. (Bar: 100μm).

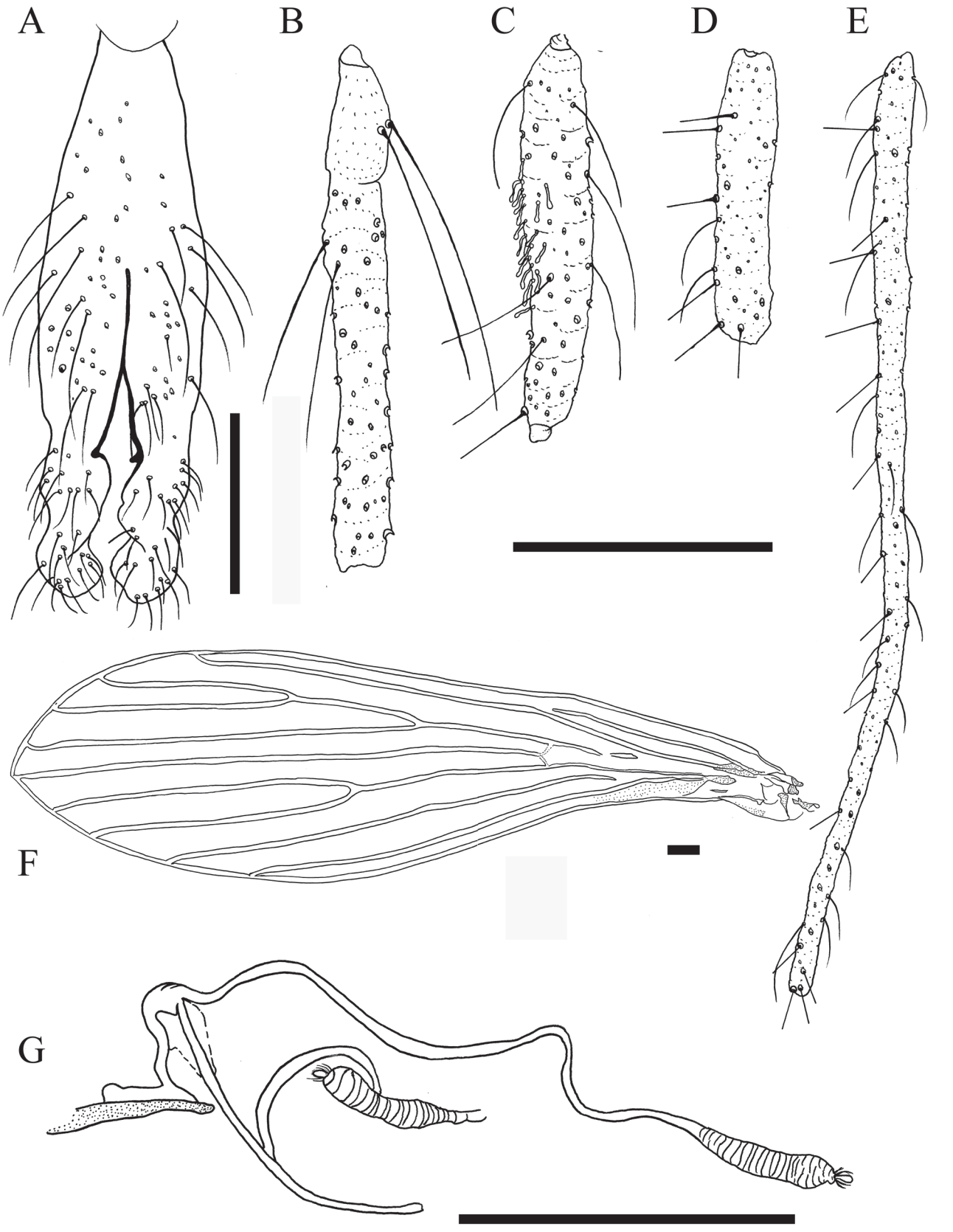

FIGURE 4. Female of *Lutzomyia* (*Lu.*) *renei* (Martins, Falcão & Silva, 1957). **A**. Labial fork. **B**. Palpus I and II. **C**. Palpus III. **D**. Palpus IV. **E**. Palpus V. (Bar: 100µm). **F**. Wing (Bar: 200µm). **G**. Spermathecae. (Bar: 100µm).

Diagnosis. Both sexes: Preapical papilla on flagellomeres I, II and III, ascoids without long posterior spur; external ascoid inserted on level more apical than the internal one. Newstead's sensilla dispersed on third palpal segment, labial suture forming a fork. Male: gonostyle with five spines: two apical, the upper external one implanted on the apical third, the lower external one more basal than the internal and this latter implanted just before the middle of the gonostyle. The dorsal margin of the paramere slightly concave and presenting in its middle two bristles with hooked apex. Gonocoxite with a basal cluster (tuft) presenting four bristles, three fine and one semi-foliaceous, implanted directly on its surface. Female: cibarium with sclerotized complete arch and absence of strong sclerotization below the posterior teeth. Two pairs of posterior teeth and several anterior teeth lateralized. 8th tergum with two to five bristles and 10th sternite with three to five apical bristles. Spermathecae with rings of equivalent length; individual spermathecal duct more than four times the length of the spermatheca and a short common spermathecal duct. Cercus *ca.* 2.0 times longer than its width.

Redescription. Male. Head 380 (370) in length. Clypeus 156 (161 and 143) long. Eyes 174 (166) long. Cibarium without teeth. Labrum-epipharynx 230 (210 and 230). Antenna (Figs 1A–D): flagellomere (F) length: F1 280 (280 and 280), FII 140 (140 and 130), FIII 140 (130), FXIII (60), FXIV (57). Only (FI–FVII) and (FI–FVIII) are present in the lectotype; paralectotypes: one of them only with FI and FII and the other with one full antenna (FI–FXIV). Ascoids: long anterior spur almost reaches the level of the preapical papilla and absence of posterior spur; external ascoid inserted on level more apical than the internal one (Figs 1A–C); antennal formula FI–FXIII 2, FXIV 0. Papilla implanted in the preapical region on FI–FIII (Figs 1A–C); presence of papillae on FXII–FXIV. Presence of simple setae on FII–FXIV. Palpus (Figs 1E–H): palpal segment (P) length: PI 42 (42 and 42), PII 133 (143 and 138), PIII 166 (161 and 179), P IV 133 (127 and 122), PV 385 (307 and 374). Palpal formula: 1-(4-2)-3-5 and 1-4-2-3-5; PII without Newstead's sensilla, PIII with several Newstead's sensilla dispersed on the middle region (Fig. 1F). Labial suture forming a fork (Fig. 1I).

Cervix. Ventro-cervical sensilla present. Cervical sclerites bearing a pair of spiniform sensilla.

Thorax. Mesonotum 470 (500 and 490) in length. Mesonotum, pronotum, paratergite, anepisternum, metanotum and postnotum brown, pleura off-white. Four proepimeral setae and seven upper anepisternal setae. Setae on the anterior margin of the katepisternum absent. Wing (Fig. 2A): 1,782 (1,822 and 1,861) long, 475 (495 and 495) wide; veins: R_5 (2,455 and 2,495); *alfa* (752 and 772); *beta* (455 and 614); *gamma* (535 and 594); *delta* (376 and 297); *pi* (238 and 337). Legs: anterior, median, posterior: coxa: 310 (300 and 290), 300 (290 and 290), 300 (300 and 300); femur: (673 and 693), 772 (594), (752 and 792); tibia: (772 and 832), 1,247 (1,010), (1,109 and 1,228); tarsomere I: (455 and 495), 732 (594), (634 and 713). Sum of tarsomeres II+III+IV+V: (475 and 594), 713 (673) and (515 and 713).

Abdomen. 1,386 (1,485) long; presence of the tergal papillae on III–VII tergum. Terminalia (Fig. 2B): gonocoxite 290 (290 and 290) long, 90 (90 and 100) wide, with basal cluster of four bristles implanted directly on its surface, three of them being fine and one semi-foliaceous (Fig. 2B and C). Gonostylus 160 (150 and 150) long, with five spines: two apical, one internal, one upper external and one lower external. The apical and upper external spines are well developed, and the lower external and internal spines are finer. However, the lower external spine is thinner than the internal one. The upper external spine is implanted at a level equidistant between the apical spines and the lower external one; this latter being implanted slightly more basally than the internal spine. Paramere: dorsal margin 159 (161 and 153) long, slightly concave in its middle region, where two bristles with hooked apex are deployed; apical half covered with spiniform setae pointing toward the base of the terminalia; ventral margin 278 (213 and 200) in length, straight with the presence of a few spiniform setae on its middle region. Parameral sheath (aedeagus) conical. Epandrium (lateral lobes) 250 (250 and 260) long, 26 (26 and 26) wide and with rounded apex. Sperm pump (ejaculatory pump) 122 (130 and 127) long; aedeagus (genital filaments) with bevel shaped apex, 570 (540 and 550) long, 4.3 times longer than the ejaculatory apodeme + sperm sac (Fig. 2D).

Female. Head 460 long, 440 wide; clypeus 156 long; eyes 221 long; Interocular distance 143 (drawings not presented). Hypopharynx with 28–30 apical teeth (Fig. 3A). Lacinia of maxilla with six external teeth and 24 internal teeth (Fig. 3B). Cibarium with four posterior teeth well-developed and 10 anterior teeth distributed in one transverse row (Fig. 3D); sclerotized area short and triangular; sclerotized arch complete. Labrum-epipharynx 360 and with 30–32 apical teeth (Fig. 3C). Antenna (Figs 3E–J): flagellomere length: FI 270, FII 120, FIII 120, FXIII 70 and FXIV 70. One antenna was missing but the other was complete (FI–FXIV). Ascoids: absence of long posterior spur; anterior spur long reaching the level of preapical papilla in FI–FXIII; external ascoids implanted more apically than the internal one; antennal formula FI–FXIII 2, FXIV 0; preapical papilla on FI–FIII (Figs 3E–

G); no papilla on FIV–FXI; papillae on FXII–FXIV. Presence of simple setae on FII–FXIV. Labial suture forming a fork (Fig. 4A). Palp (Fig. 4B–E): palp length: PI 65, PII 187, PIII 200, PIV 143 and PV 468. Papal formula: 1-4-2-3-5; Newstead's sensilla absent on PII; PIII with Newstead's sensilla dispersed on its middle region (Fig.4C).

Cervix. Ventro-cervical sensilla present. Cervical sclerites bearing paired spiniform sensilla.

Thorax. Mesonotum 330 in length. Mesonotum, pronotum, paratergite, anepisternum, metanotum and postnotum brown, pleura off-white. Four proepimeral setae; seven upper anepisternal setae. Setae absent on the anterior region of the katepisternum. Wing (Fig. 4F): 2,356 long, 653 wide; veins: R_5 1,822; *alfa* 594; *beta* 376; *gamma* 535; *delta* 297; *pi* 198. Legs: anterior; median; posterior: coxa: 713; 673; 693; femur: 812; 812; 950; tibia: 950; 1,168; 1,465; tarsomere I: 594; 693; 832. Sum of tarsomeres II+III+IV+V: 733; 752; 871.

Abdomen. 1,782 long; 8th tergum with two to five bristles and 10th sternite with three to five apical bristles. Spermathecae 52 long and 13 wide, ringed with rings of equivalent length (Fig. 4G): 52; common spermathecal duct 31 long and 10.4 wide; individual spermathecal ducts 174 long and 5.2 wide. The individual and the common spermathecal ducts are membranous and with smooth walls. Cercus *ca.* 2.0 times longer than wide.

Material examined. Type series of *Lu. renei* deposited in the "Coleção de Referência Nacional e Internacional de Flebotomíneos, Centro de Pesquisas René Rachou" (CRNIF–CPqRR) with the slides numbered as follows: "cotype" n°1 collected on 10-III-1957 (1 ♂), "cotype" n°164 collected on 24-XI-1955 (1 ♂), "cotype" n°165 collected on 24-11-1955 (1 ♂) and n°1,087 (1 ♀). According to the original description, all the "cotypes" were deposited in the collection of "Instituto Nacional de Endemias Rurais", Belo Horizonte municipality, Minas Gerais state, currently CPqRR-FIOCRUZ. Only three specimens are still deposited in that institution: the specimen collected on 10-III-1955 was designated lectotype, and the two specimens collected on 24-XI-1955 paralectotypes. The remaining three specimens, if found, should also be designated paralectotypes.

Distribution. BRAZIL: Martins *et al*. (1978, p.25). MINAS GERAIS: Uberlândia, Lemos *et al*. (2004, p.195); Varzelândia, Andrade *et al*. (2007, p.981); Parque Nacional Cavernas do Peruaçu, Barata *et al*. (2008, p.226); Diamantina, Barata & Apolinário (2012, p.1017); Lassance, Carvalho *et al*. (2012, p.3). TOCANTINS: Arraias municipality previously belonging to the state of GOIÁS, Martins *et al*. (1978, p.25). MATO GROSSO DO SUL: Campo Grande, Oliveira *et al*. (2003, p.936); Silva *et al*. (2007, p.422).

Medical importance. Coelho & Falcão (1962) demonstrated under laboratory conditions that *Lu. renei* transmited *Leishmania mexicana* Garnham, 1962, cited back then as *Leishmania braziliensis* by these authors. Later on, Coelho *et al*. (1967a–b) conducted studies of experimental infections of *Leishmania* sp., however, there is doubt as to whether the species used was *Le. braziliensis* or *Le. mexicana* (Killick-Kendrick 1986). Gontijo *et al*. (1987) experimentally infected one male of *Lu. renei* with *Leishmania* sp. Currently there is no evidence implicating *Lu. renei* as a vector of *Leishmania* spp.

Discussion

Martins *et al*. (1957) grouped *Lu. renei* with other species that have inserted on the dorsal margin of their parameres two bristles with hooked apices as well as having segment V of the palpus longer than the others. These authors considered *Lu. longipalpis*, *Lu. gaminarai* and *Lu. cruzi* closely related to *Lu. renei,* the latter distinguished by the presence on the gonostyle of two well developed apical spines while there is only one in the other species. *Lutzomyia renei* was described as having on the gonocoxite, a cluster of four long setae, three of them foliaceous. However, in the "cotypes" analyzed here, we found three fine (as wide as the genital filaments) bristles and one semi-foliaceous (twice as wide as the fine bristles). Sherlock (1957) described the female of *Lu. renei* and highlighted the similarity of its annulated spermathecae to those of *Nyssomyia whitmani* and *Lu. longipalpis*. The female of *Lu. renei* was distinguished from that of *Ny. whitmani* by the length of the palpus, head and spermathecae and from that of *Lu. longipalpis* because the latter presents shorter spermathecae and individual spermathecal ducts as well as having the apical annuli of the spermathecae presents a smaller to that of the others.

Based on the classification of Galati (2003, 2015), *Lu. renei* has morphological characters that place it, along with 20 other species, in subgenus *Lutzomyia s. str.* Males of this group have gonostyle with the lower external spine implanted at a more basal level than the internal one; one or two apical spines, in the former case, the preapical spiniform seta is generally present; inserted on the dorsal margin of the paramere are bristles with hooked apices. Pertinent characters of the females include a cibarium with the sclerotized arch complete and the anterior

teeth in vertical position and/or lateralized; hypopharynx with teeth well defined; 8th tergum with two to five bristles and three to five apical bristles in the 10th sternite; spermathecae ringed, short common spermathecal duct and long individual spermathecal ducts; this latter being *ca.* 4.0 times longer than the spermathecae or the common spermathecal duct. Both sexes exhibit palpus II equivalent in length to palpus IV and palpus V longer than III; flagellomeres FI–FIII with pre apical papilla and no setae on the anterior region of the katepisternum.

Lutzomyia renei may be distinguished from other species of the subgenus by both male and female characteristics as well as some found in both sexes. Males and females of *Lu. renei* may be distinguished from those of *Lu. dispar* and *Lu. fonsecai* by the absence of the labial fork in the latter two species. Regarding males of the subgenus, *Lu. renei* may be differentiated from those of *Lu. almerioi*, *Lu. forattinii* and *Lu. elizabethrangelae* due to the presence in these species of foliaceous bristles implanted in a tubercule, on the base of the gonocoxite; from those of *Lu. alencari*, *Lu. bifoliata*, *Lu. cruzi*, *Lu. falquetoi*, *Lu. gaminarai*, *Lu. ischnacantha*, *Lu. ischyracantha*, *Lu. lichyi*, *Lu. longipalpis*, *Lu. matiasi*, *Lu. pseudolongipalpis* and *Lu. souzalopesi*, because these species present only one apical spine; from that of *Lu. battistinii* because this species presents five to six bristles with hooked apices on dorsal margin of the paramere; from that of *Lutzomyia bicornuta* because in this species the bristles of the basal cluster of the gonocoxite are implanted in a tubercle, and from that of *Lu. cavernicola* due to the presence in the latter species of a gonostyle with the lower external and internal spines equally developed and the ventral margin of the paramere tapers abruptly in its apical third.

In females of the subgenus, the apical ring of the spermathecae being longer than the pre-apical one, distinguishes *Lu. battistinii*, *Lu. bicornuta* ,*Lu. ischnacantha, Lu. lichyi*, *Lu. bifoliata* and *Lu. cavernicola* (Young & Duncan 1994, Galati 2003, 2015) from *Lu. renei*. Moreover, the number of posterior teeth of the cibarium differ among species, with three or more pairs in *Lu. pseudolongipalpis*, *Lu. longipalpis, Lu. cruzi*, *Lu. gaminarai*, *Lu. matiasi*, *Lu. ischyracantha* and *Lu. alencari* differing from the ## found in *Lu. renei*. *Lu. souzalopesi* with the cercus *ca.* 4.0 times longer than it is wide distinguishes it from *Lu. renei*. *Lutzomyia almerioi*, with a sclerotized protuberance on the epandrium (9th tergum), is easily differentiated from *Lu. renei.* The presence of three to five apical bristles on the 10th sternite in *Lu. renei* permits its distinction from *Lu. forattinii* and *Lu. elizabethrangelae* which have only two apical bristles.

As a result of carefully reviewing and redescribing the morphology of *Lu. renei*, this species may be distinguished from all the others included in subgenus *Lutzomyia*. Sound taxonomy is critical to the success of entomological surveillance and research on sand flies as potential vectors of disease agents. Our study highlights the importance of taxonomic reviews in assuring the correct identification of species.

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